SY-5609, an orally available selective CDK7 inhibitor, demonstrates broad anti-tumor activity in vivo

**Abstract**

Previously, we reported on a series of highly potent, selective, and non-covalent CDK7 inhibitors that demonstrated antiproliferative activity against triple-negative breast cancer (TNBC) and ovarian cancer (OVA) cell lines and tumor growth inhibition in cell line-derived (CDX) and patient-derived (PDO) mouse xenograft models. Here, we report on the in vitro and in vivo profile of our development candidate, SY-5609.

**Methods:** Kinase inhibition assays at both Kc and 2 μM (ATP) were used to assess inhibition of CDK7, CDK9, CDK12, and CDK14. SPR was used to determine the Kd, Kc, and binding characteristics of SY-5609 to immobilized CDK7/Cyclin H dimers. CDK7 compound occupancy was determined using a biotinylated small molecule probe to pull down free CDK7 following incubation of HL60 cells with SY-5609. Kinase inhibition assays at both 25 and 50 nM were determined using a biotinylated small molecule probe to pull down free CDK7 following 72 hrs of incubation with SY-5609. Flow cytometry was used to assess apoptosis and cell cycle modulation after 48 hrs of treatment. CDK7 kinase activity was determined using a biotinylated small molecule probe to pull down free CDK7 following incubation of HL60 cells with SY-5609. Absolute tumor growth inhibition (TGI) was determined using a biotinylated small molecule probe to pull down free CDK7 following incubation of HL60 cells with SY-5609. Flow cytometry was used to assess apoptosis and cell cycle modulation after 48 hrs of treatment. DNA damage repair was assessed by immunofluorescence staining for γH2AX and RAD51 proteins. To assess in vivo effects, mice were implanted subcutaneously and randomized for treatment when tumors reached 150-200 mm3 and dosed orally for 3 weeks by both QD and BD dosing regimens. Collected tumor tissue samples were analyzed for protein levels of MCL1, pCDK2, MYC, and downstream markers of CDK7 activity.

**Results:** SY-5609 bound CDK7/Cyclin H with a Kd of 0.009 nM and occupied CDK7 in HL60 cells with an EC50 of 0.17 nM in a panel of solid tumor cell lines. Selectivity of SY-5609 over CDK2, CDK12, and CDK14 was 13,000-, 16,000-, and 49,000-fold, respectively. SY-5609 led to induction of apoptosis, cell cycle arrest, and inhibition of DNA repair in tumor cell lines. Significant growth inhibition was observed in a panel of CDX and PDO solid tumor models with both QD and BD dosing of SY-5609 with resulting decreases in direct (pCDK2, RNA Pol II CTD pS2 phosphorylation) and indirect (MCL1, MYC, CDK7) protein biomarkers.

In summary, we describe SY-5609, an orally available, potent, and selective CDK7 inhibitor that drives robust TGI as well as inhibition of downstream CDK7 markers in CDX and PDO tumor models. These data support the rationale for advancing SY-5609 into IND-enabling studies.

**Conclusions**

- SY-5609 is a potent, selective oral CDK7 inhibitor that demonstrates broad anti-tumor activity in vivo.
- SY-5609 is well tolerated in multiple PDX models with complete tumor regressions as a monotherapy observed in multiple TNBC and OVA models.
- SY-5609 demonstrates substantial tumor growth inhibition and is well tolerated in multiple PDX models.
- SY-5609 in vivo activity is associated with selective inhibition of CDK7 and downstream cell cycle markers.
- SY-5609 is well tolerated in multiple PDX models with complete tumor regressions as a monotherapy observed in multiple TNBC and OVA models.
- SY-5609 is being progressed through IND-enabling studies to support initiation of a Phase 1 oncology trial in early 2020.